

# Analytical statistical interpretation of relationship between different parameters of kiwifruit (*Actinidia deliciosa* cv. Hayward) during cold storage

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**Key words:** ascorbic acid, correlation, kiwifruit, total antioxidant activity, total phenolic compounds.

**Abstract:** Physicochemical and metabolic changes of kiwifruit characteristics were studied during storage. Parameters, such as total phenolic compounds (TPC), color ( $L^*$ , hue, chroma), total antioxidant activity (TAA) and ascorbic acid (AA) content, were evaluated at harvest and every 15 days during fruit storage at 0°C and 90%  $\pm$  5 RH. Correlations between different parameters were also evaluated. The results of this study suggest that high antioxidant capacity in kiwifruit is due to a strong association ( $r^2 > 0.90$ ) between AA and TPC, and that phenolic compounds and AA are the major contributors to the antioxidative activities of *Actinidia deliciosa* cv. Hayward. Color parameters were found to have weak correlation coefficients with TPC, TAA and AA. It is worth noting that the physical-chemical parameters do not have a relationship with the chromatic parameters.

## 1. Introduction

Kiwifruit (*Actinidia deliciosa*) is cultivated mainly due to its sensory properties and its ability for prolonged cold storage. This latter attribute enables consumption of kiwifruit throughout the year in many parts of the world (Minas *et al.*, 2010). The most widely grown *Actinidia* cultivar is the *A. deliciosa* cv. Hayward. Commercial production of this variety has spread to many countries because of its distinctive characteristics, including fruit size, high productivity, and sufficient storageability (Ferguson, 1999).

Kiwifruit is a good source of natural antioxidant substances, in particular vitamin C (Nishiyama *et al.*, 2004). In fact, the content of vitamin C in kiwifruit ranges between 25 and 155 mg/100 g of fresh weight (FW) of fruit (Tavarini *et al.*, 2009), making it higher than that determined in orange, strawberry, lemon and grapefruit. Beever and Hopkirk (1990) showed that vitamin C content in kiwifruit was tenfold higher than the same content found in apple and peach. Esti *et al.* (1998) have observed that the vitamin C content of kiwifruit depends on genotype, ripening degree, storage and the analysis method utilized. Kiwifruit's strong antioxidant capacity is due to a wide number of phytonutrients including carotenoids, lutein, phenolics, flavonoids and chlorophyll (Kaya *et al.*, 2008). In fact, during fruit ripening, several biochemical, physiological

and structural modifications occur and these changes determine the final fruit quality attributes (Lee and Kader, 2000; Ayala-Zavala *et al.*, 2004).

During postharvest storage of horticultural crops, important changes in antioxidant status can occur (Ayala-Zavala *et al.*, 2004). Kiwifruit is rich in bioactive compounds, especially polyphenols (Park *et al.*, 2009). Polyphenolic compounds are a complex group of substances that have gained enormous attention in recent years, especially within the analytical chemistry field because they exhibit important quality properties and antioxidant activity (Escarpa and González, 2001). The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides (Osawa, 1994).

The dark green color of kiwifruit, due to the chlorophylls in plastids in the pericarp cells of the flesh (Talens *et al.*, 2002), can be modified during storage. Most fleshy fruits are green only during the earlier stages of development: they undergo dramatic changes in chemical composition and ultra-structure during maturation and ripening. Associated with tissue softening and the changes in carbohydrate and organic acid metabolism there is conversion of chloroplasts into chromoplasts and a concomitant loss of chlorophyll, often accompanied by accumulation of carotenoids (Montefiori *et al.*, 2009). The pigments responsible for the flesh color in ripe fruit of *A. deliciosa* have already been described (McGhie and Ainge, 2002; Nishiyama *et*

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Received for publication 25 June 2014

Accepted for publication 17 September 2014

al., 2005); the color is mainly due to chlorophylls a and b (Nishiyama *et al.*, 2005).

The changes in bioactive compounds of kiwifruit that occur during cold storage have not previously been studied, thus in this paper we describe changes in pericarp color, ascorbic acid (AA), total antioxidant activity (TAA) and total phenolic contents (TPC) in the fruits with different storage times. The differences and correlations between these parameters are also examined.

## 2. Materials and Methods

### Plant material

Mature, unripe kiwifruit (*Actinidia deliciosa* cv. Hayward) of medium size (80-120 g), free from visible defects or decay, were harvested from a commercial kiwifruit orchard in Gorgan, Iran with average firmness of 10 (kg/cm<sup>2</sup>) and 7% °Brix. Fruits were immediately transferred to the postharvest laboratory at Shiraz University. Kiwifruits were individually labeled and packaged into ventilated bags, then stored for four months at 0±1°C and 90±5% relative humidity (RH). Samples were taken at monthly intervals during storage for quality evaluation and analyses.

### Physical and physicochemical assays

**Total phenolic contents.** The total phenolic contents of each extract were determined according to the method of Gutfinger (1981). Extracts (1 ml) at 1 mg/ml concentration were mixed with 1 ml of 2% Na<sub>2</sub>CO<sub>3</sub>. After standing for 3 min, 0.2 ml of 50% Folin-Ciocalteu reagent was added to the mixture and left to stand for 30 min. The mixture was centrifuged at 13 400×g for 5 min. The absorbance was measured at 750 nm and TPC are expressed as gallic acid equivalents (GAE).

**Color.** Color was determined using digital imaging (Afshari-Jouybari and Farahnaky, 2011). Fruit was photographed in a chamber; angle light with the horizontal surface of the images was 45 degrees. After transferring the images to a computer, Photoshop image processing software was performed. Individual *L\**, *a\** and *b\** parameters were recorded: *L\** is lightness, *a\** [-greenness to +redness) and *b\** (-blueness to + yellowness) are chromacity coordinates. The *a\** and *b\** values were converted to chroma ( $C^* = (a^{*2} + b^{*2})^{1/2}$ ) and hue angle ( $h^{\circ} = \tan^{-1}(b^*/a^*)$ ).

**DPPH radical scavenging activity (RSA).** The free radical scavenging activity was measured by 2,2-diphenyl-2-picrylhydrazil (DPPH) on the basis of the method of Brand-Williams *et al.* (1995) with minor modifications. For this determination, aliquots (0.1 mL) of the extract were mixed with 1 mL of a DPPH solution (500 µM) in 80% ethanol. The mixture was incubated at room temperature for 30 min. Solution absorbance was determined at λ =515 nm. The DPPH radical concentration was calculated using the following equation: scavenging effect (TAA %) = (1 - Af/Ao) × 100; Ao stands for the absorbance of the control sample and Af for the absorbance in the presence of the sample. L-ascorbic acid was used for the calibration curve, and the results are expressed as mg L-ascorbic acid equivalent. 100 g<sup>-1</sup> fw (fresh weight).

**Ascorbic acid.** Ascorbic acid was measured by the oxidation of ascorbic acid with 2, 6-dichlorophenol endophenol and the results are expressed as mg/100 g fresh weight (Rangana, 1977).

**Statistical analysis.** Four replicates of each sample were used for statistical analysis. Correlation analyses between different parameters were carried out using the correlation and regression programme in MINITAB 16. Correlations were obtained by Pearson's correlation coefficient (r) in bivariate linear correlations. All statistical analyses and correlations were carried out with SAS software package v. 9.1 for Windows. Differences at P<0.05 were considered to be significant.

## 3. Results and Discussions

### Ascorbic acid

Ascorbic acid (AA) content first increased up to 30 days (47.74 mg/100 g<sup>-1</sup>) and then decreased (Table 1) and could be a result of its synthesis during the initial storage period. The observed variation (increase) of AA content is due to fruit weight loss by dehydration (hence due to a higher concentration) or actually to AA *ex novo* synthesis. Utilization of AA during later storage periods may be the reason for its decreased amounts. The accumulation of AA during ripening depends on the type of fruit. Lee and Kader (2000) reported that AA content increased with ripening in apricot, peach and papaya, but decreased in apple and mango. Generally, when fruits become overripe, vitamin C

Table 1 - Changes in AA, TPC, TAA and chromacity of kiwifruit during storage

Storage time (day)	Ascorbic acid (mg/100 g FW)	Total phenolic compounds (mg/100 g DW)	Total antioxidant activity (mg/ 100 g FW)	Color parameters		
				L*	Hue	Chroma
0	40.17±1.53 b	45.75 ±0.99 c	489.17±42.01 bc	51.9 ± 0.83 a	66.92±1.01 a	48.42±1.75 a
30	47.74 ±1.38 a	57.9±1.91 a	602.17±43.97 a	46.07±0.55 b	64.63±2.95 ab	45.49±1.19 b
60	40.97±2.17 b	50.65±3.35 b	556.20±70.33 ba	41.34±0.78 c	62.20±0.73 bc	39.61±0.77 c
90	35.68±2.11 c	43.3±2.56 c	434.34±47.60 dc	35.35±1.50 d	61.82±1.33 bc	35.68±1.16 d
120	27.44±1.89 d	30.45±1.65 d	354.18±40.84 d	39.92±0.82 c	59.76±1.41 c	32.62±1.73 e

Means within each column with different superscript letters are significantly different (p < 0.05) for each sampling.

content declines concurrently with the degradation of fruit tissues (Kalt, 2006).

In persimmon fruits, AA and TPC content showed a linear relationship with a positive correlation coefficient of  $r^2=0.976$  (Fig. 1). Other authors found strong correlations between AA and TPC in different fruits (Gonçalves *et al.*, 2004; Serrano *et al.*, 2005). Ascorbic acid content was positively correlated with antioxidant activity ( $r^2 = 0.944$ ), suggesting that AA makes a significant contribution to the total antioxidant capacity of kiwifruit (Fig. 2).

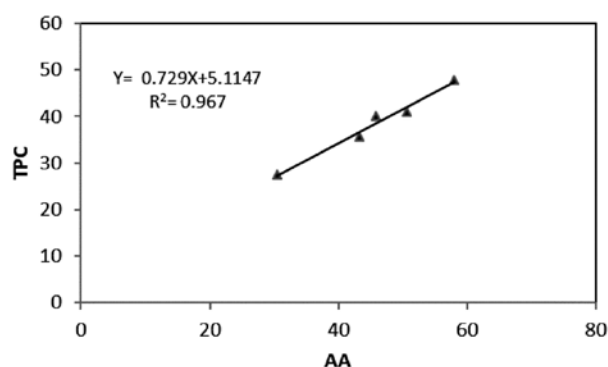


Fig. 1 - Correlation between TAA and AA in kiwifruit.

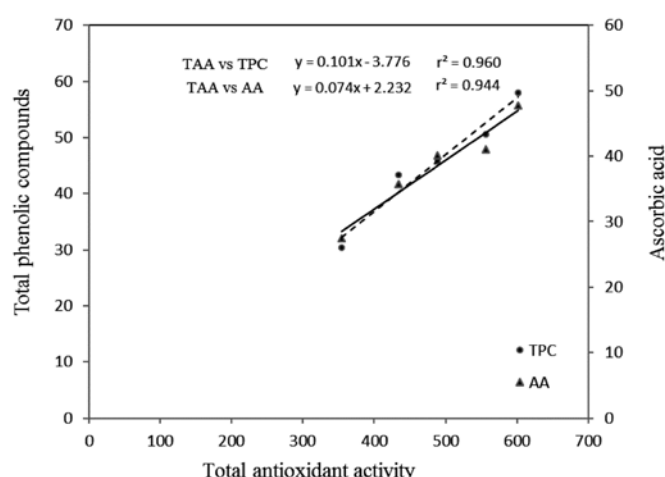


Fig. 2 - Correlation between TAA and total phenolic compounds or ascorbic acid in kiwifruit.

### Total phenolic compounds

Total phenolic compounds of fruits increased up to 30 days ( $57.9 \text{ mg}/100 \text{ g}^{-1}$ ) and then decreased (Table 1). During storage, TPC increased initially, probably due to synthesis from sugars, and decreased later due to its participation or utilization in other metabolic processes. Tavarini *et al.* (2008) reported that TPC may increase or decrease in fruits and vegetables, depending on the storage conditions. The increase in TPC during storage may be the result of fruit damage and tissue disruption during storage. Phenolic compound synthesis in response to wounding has been reported (Saltveit, 2000).

All o-quinone molecules are highly reactive and may interact with other phenols or other substances, co-poly-

merise and thus produce compounds, which determine undesired fruit browning, or oxidise further compounds, reduce to original phenols, or react with different nucleophile compounds such as amines, thiols, imidazole, and indole. Generally there is a positive correlation between TPC availability and vulnerability to PPO attack (Ramírez *et al.*, 2003). Phenolic compounds represent the main substrates used by oxidative enzymes, having consequences in terms of color and quality changes, as well as being associated with plant defense mechanisms against stress situations that can affect the postharvest period (Tomás-Barberán and Espín, 2001).

Good correlation between TPC and AA was observed with a high significance level ( $P<0.001$ ), and a similar relationship (0.97) was also obtained between TPC and TAA (Table 2). This positive and significant relationship between TPC and TAA was greater compared to AA and TAA. The results indicate strong association between antioxidative activities and phenolic compounds, suggesting that the latter are probably responsible for the antioxidative activities of kiwifruit. Phenolic compounds are also effective hydrogen donors, making them good antioxidants (Rice-Evans *et al.*, 1995). Reports in literature on the relationship between TPC and TAA are contradictory; some authors have observed a high correlation (Proteggente *et al.*, 2002; Tsao *et al.*, 2003; Khanizadeh *et al.*, 2008).

Table 2 - Correlation matrix (Pearson correlation coefficients)

	TPC	AA	TAA	Chroma	Hue	L*
TPC	1.00					
AA	0.98***	1.00				
TAA	0.97**	0.97**	1.00			
Chroma	NS	NS	NS	1.00		
Hue	NS	NS	NS	0.97**	1.00	
L*	NS	NS	NS	0.89*	0.86*	1.00

NS= not significant.

\* Significant to 0.05  $p$  level.

\*\* Significant to 0.01  $p$  level.

\*\*\* Significant to 0.001  $p$  level.

### Total antioxidant activity

TAA increased and peaked over the course of one month ( $57.9 \text{ mg}/100 \text{ g}^{-1}$ ) then decreased toward the end of the storage period (Table 1). There is debate in the literature about the influence of vitamin C on the antioxidant capacity of fruits and vegetables (Guo *et al.*, 2003). However, it is also known that fruits with high antioxidant capacity generally contain more antioxidants and most of these antioxidants have been shown to be phenolic compounds, in particular flavonoids (Guo *et al.*, 2003). For example in pomegranate, ascorbic acid and phenolic compounds are responsible for the TAA, alone or in combination (Kulkarni and Aradhya, 2005). Examining the entire storage period, it can be observed that changes in antioxidant activity

were very similar to phenolic compounds (Lemoine *et al.*, 2009). TAA was highly and positively related to AA ( $r^2 = 0.944$ ) (Fig. 2). However, in the present study, the best correlation ( $r^2 > 0.96$ ) was observed between TPC and RSA (Fig. 2). This fact probably indicates that the antioxidant capacity of kiwifruits is primarily due to TPC and AA.

### Color

A significant decrease in hue angle was observed during the storage of fruits at 0°C, indicating continued ripening during cold storage. The initial L\* value was 51.9. Internal lightness decreased sharply within 90 days of storage and then increased significantly until the end of storage (Table 1). The chroma (C\*) value was initially 48.42 (Table 1). Chroma values (internal) of fruits decreased during the entire storage period and reached their minimum values at the end of storage. Changes in C\* result principally from a loss of chlorophyll content, mostly chlorophyll *a* which decreases during storage (Fukey *et al.*, 1985). In kiwifruit, lightness and chroma values significantly decreased during cold storage and shelf-life, indicating less color intensity (Koukounaras and Sfakiotakis, 2007). When fruits darken, skin color becomes less chromatic and surface browning develops. Diminished red skin and darkening due to oxidative browning reactions have been found to be more marked in ripe strawberries that suffer greater moisture loss during storage (Nunes *et al.*, 2005).

Correlation analyses showed that in the fruit tissues, most of the correlation coefficients were lower, positive and not significant. However, the correlation between chroma of kiwifruit and TPC, TAA and AA was weakly positive and significant (Table 2). These results indicate that the physical-chemical parameters do not have a relationship with the chromatic parameters and TAA, TPC and AA in kiwifruit. Similar results were reported by Drogoudi *et al.* (2008) and Vieira *et al.* (2009).

### 4. Conclusions

Taken together, the results of this study imply that high antioxidant capacity in kiwifruit is due to a strong association between AA and TPC; color parameters of kiwifruit were found to have high correlation coefficients with TPC. It is worth emphasizing that these latter have been correlated with the degree of browning as a result of decreased antioxidative activities.

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